# Condition and diet of larval *Pleuragramma antarcticum* (Nototheniidae) from Terre Adélie (Antarctica) during summer

by

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**ABSTRACT**. - During austral summer 1996, the ichthyoplankton was sampled near the coast of Terre Adélie. *Pleuragramma antarcticum* Boulenger, 1902 constituted 99% of the sampled larvae. Larval fish condition was investigated by histology of the digestive organs. Most of the larvae were in good condition or slightly starved, some of them were severely starved but none were at a point of no-return. It seems that larvae feeding mainly on copepods are in better condition than those feeding mainly on diatoms, but also that those having an omnivorous diet are in better condition. There was no evidence for a build-up of body reserves allowing to sustain the larvae over winter.

**RÉSUMÉ**. - Condition physiologique et régime alimentaire des larves de *Pleuragramma antarcticum* (Nototheniidae) en Terre Adélie (Antarctique) pendant l'été.

Durant l'été 1996, des prélèvements d'ichtyoplancton ont été effectués dans la zone côtière de Terre Adélie. Quatre-vingt-dix-neuf pour cent des larves appartenaient à *Pleuragramma antarcticum* Boulenger, 1902. Des études histologiques ont été effectuées afin de déterminer leur condition physiologique. Si la plupart des larves étaient en bonne condition ou avaient subi un jeûne léger, certaines ont montré des signes de jeûne sévère. Nous avons essayé de déterminer si le régime alimentaire pouvait expliquer les différences de condition larvaire. Il semble que, non seulement les larves se nourrissant principalement de copépodes seraient en meilleure condition que les larves ayant un régime alimentaire à base de diatomées, mais qu'aussi les larves ayant un régime alimentaire omnivore seraient dans de meilleures conditions. Des conclusions sont données sur la survie de ces larves à long terme sachant qu'aucune réserve n'a été observée.

Key words. - Nototheniidae - *Pleuragramma antarcticum* - Southern Ocean - Larvae - Diet - Condition index.

During the first French ichthyoplanktonic survey in Terre Adélie (Antarctica), in January and February 1996, the larvae of *Pleuragramma antarcticum* Boulenger, 1902 were the most abundant species (Koubbi *et al.*, 1997). A large shoal of larval *P. antarcticum* was observed in the coastal zone. Larval concentration was correlated with high temperatures. This species occupies the coastal pelagic niche, thus avoiding interspecific competition with other fish and intraspecific competition between larvae and older fish (Hubold and Ekau, 1987). Larval *P. antarcticum* are found in the coastal zone whereas juveniles and adults are associated with the shelf-break.

From the match/mismatch hypothesis (Cushing, 1975), we know that larval development should coincide spatially and temporally with plankton blooms. Moreover, Hjort (1914) deduced that there is a critical period at the end of the endogenous nutrition (on vitelline reserves) and at the beginning of the exogenous feeding. At this stage, if larvae do not find their prey, they may starve and reach the point of no-return. This means that even after feeding, the larvae will not recover and will not survive (Blaxter and Hempel, 1963). *P. antarcticum* has a long pelagic larval phase of over one year (Kellermann, 1987). The larvae have to survive the critical period and also through winter when planktonic resources are low (Longhurst, 1998). Consequently, it is important to determine if the plankton bloom will enable larval fish survival or not.

Diet contents of fish larvae are usually observed by light microscopy. However, Scanning Electron Microscope (SEM) has recently been widely used to perform study on gut microflora (Jolly *et al.*, 1993; Mendez *et al.*, 2003). This method enables the determination of the vacuity of fish larvae guts and the composition of their diet more precisely.

Beside gut contents, it is important to determine the condition of larvae. Different techniques can be used to determine larval condition, namely morphology, histology and

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biochemistry (DNA/RNA index, enzymology). Many authors (Suthers, 1993; Ferron and Leggett, 1994; Theilacker and Porter, 1994) concluded that histology is the most accurate because it shows the direct consequences of starvation on organs and because it is less affected by temperature or larval age.

Boulhic (1991) proposed a method adapted from O'Connell (1976) to determine the condition of the Dover sole larvae by histology. This method is based on the observation of the foregut, midgut, hindgut, liver and pancreas.

The aim of the present study was to assess the likely survival of *P. antarcticum* larvae by investigating their nutritional status in relation to the environment using microscopical techniques and condition indices linked to starvation or nutritional status.

## MATERIAL AND METHODS

### Sampling

During January and February 1996, 33 stations (Fig. 1) were sampled in the coastal zone of the Pierre Lejay Bay in Terre Adélie (Antarctica) (Koubbi *et al.*, 1997). Ichthyoplankton was collected by oblique tows of a bongo net (500 µm) from the surface to the bottom or to a maximum depth of 200 m. Fish larvae were preserved in 5% seawater buffered formalin. Some larvae were fixed in Bouin Holland solution for histology. Mesozooplankton was also collected with vertical hauls of a WP2 (200 µm).

### Histology

Thirty-six larval *P. antarcticum* were histologically processed to determine their conditions. Their standard lengths varied between 15 and 30 mm (mean SL 18.7 mm). Five larvae were from stations 85-86 (January the 18th and 19th, 1996), 17 from station 72 (January the 31st, 1996) and 14 from station 53 (February the 3rd, 1996).

Larvae were fixed in Bouin Holland solution for 48 hours immediately after capture. Then, stored in 70° alcohol. Dehydration was in 95% and absolute alcohol before embedding in paraffin wax (Grioche, 1998). Sections of 7µm thickness were taken using a standard microtome. The following stains were applied: blue alcian for acid mucopolysaccharids, Groat-hematoxyline for the nucleus, and orange G for general tissue (Martoja and Martoja-Pierson, 1967).

### Larval condition

Cell and nucleus morphology, intercellular aspect or presence of secretions or vacuoles for each organ was noted (O'Connell, 1976; Boulhic, 1991; Grioche, 1998) to define the condition of fish larvae from grades 1 to 6:

Grade 1: Point of no-return inducing eventual death. Nuclei are pycnotic. Tissues of liver, gut and pancreas are severely damaged and will never recover to a normal state;

Grade 2: Severe starvation. All organs are affected, which is shown by the poor intercellular cohesion of many organs. Hepatocytes are small. Zymogen is absent from the pancreas;

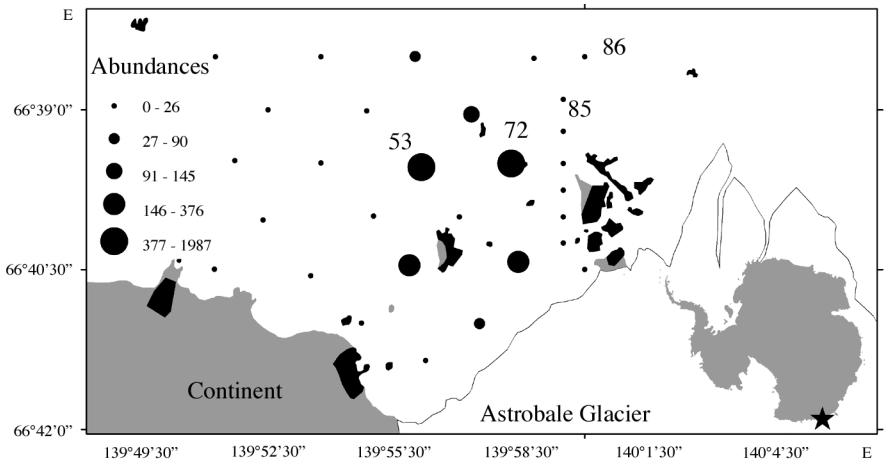


Figure 1. - Location of stations in the Pierre Lejay Bay (Terre Adélie) during summer 1996. Abundance of *Pleuragramma antarcticum* larvae (number of individuals per 100 $m^3$). Stations number where larvae were sampled for condition indices are given. Stations 85 and 86 were sampled on the 18th and 19th of January, station 72 on 31st of January and station 53 on the 3rd of February. Abundance classes were created by using the natural breaks classification of ArcGis which allows better visualisations of abundance break values. *[Localisation des stations dans la baie Pierre Lejay (Terre Adélie) pendant l'été 1996. Abondance des larves de* P. antarcticum *(nombre d'individus pour 100 $m^3$). Les numéros de stations où les indices de condition larvaire ont été relevés sont donnés. Les stations 85 et 86 ont été échantillonnées les 18 et 19 janvier, la station 72 le 31 janvier et la station 53 le 3 février. Les classes d'abondance ont été définies en utilisant la méthode de classification fondée sur les seuils naturels d'ArcGis, ce qui permet de mieux visualiser les valeurs des sauts d'abondance.]*

Table I. - Histologic categories and codes for determining larval conditions based on observation of cells and tissues. Per line of the table, only one category can be chosen per larvae for the multivariate analysis. *[Catégories histologiques et codes déterminant les conditions lar - vaires fondés sur les observations des tissus et cellules. Par ligne du tableau, seulement une catégorie peut être choisie par larve pour l'analyse multivariée.]*

| | Feature | Categories and codes for the multivariate analysis |
|---|---|---|
| Foregut | *Caliciform cells* | Absent (CC0); few (CC1); some (CC2); numerous (CC3) |
| Midgut | *Enterocyte* | |
| | Shape of the cell | Cube-shaped (EIM1); High (EIM2); Very high (EIM3) |
| | Cytoplasm density | Clear (CYIM1); Full (CYIM2); Dense (CYIM3) |
| | Nucleus | Small or irregular (NIM1); Circular or large (NIM2) |
| | Vacuoles | Absent; Present |
| | *Intercellular cohesion* | Many intercellular gaps (CIM0); Some intercellular gaps (CIM1); Good cell juxtaposition (CIM2); Perfect cell juxtaposition (CIM3) |
| Hindgut | *Enterocyte* | |
| | Shape of the cell | Cube-shaped (EIP1); High (EIP2); Very high (EIP3) |
| | Cytoplasm density | Clear (CYIP1); Full (CYIP2); Dense (CYIP3) |
| | Nucleus | Small or irregular (NIP1); Circular or large (NIP2) |
| | Vacuoles | Absent (GIP0); Present (GIP1); numerous (GIP2) |
| | *Intercellular cohesion* | Many intercellular gaps (CIP0); Some intercellular gaps (CIP1); Good cell juxtaposition (CIP2); Perfect cell juxtaposition (CIP3) |
| Liver | *Hépatocyte* | |
| | Shape of the cell | Irregular (CF1); Normal (CF2); Large (CF3) |
| | Nucleus | Small or irregular (NF1); Circular or large (NF2) |
| | Vacuoles | Absent; Present |
| | *Intercellular cohesion* | Many intercellular gaps; Some intercellular gaps (CICF1); Good cell juxtaposition (CICF2); Perfect cell juxtaposition (CICF3) |
| Pancreas | *Cell* | |
| | Shape of the cell | Irregular (CP1); Normal (CP2); Large (CP3) |
| | Nucleus | Small or irregular (NP1); Circular or large (NP2) |
| | Zymogen | Absent (Z0); Present (Z1); Numerous (Z2); High density (Z3) |
| | *Acinis cohesion* | Many intercellular gaps (CA0); Some intercellular gaps (CA1); Good cell juxtaposition (CA2); Perfect cell juxtaposition (CA3) |

Grade 3: Starvation or feeding after severe starvation. Gut and liver are affected and intercellular gaps are observed. Gut cells have a reduced height;

Grade 4: Beginning of starvation but signs of digestive absorption. One organ may be affected. Few or no vacuoles are found in the hindgut;

Grade 5: Good condition even if no recent feeding. No hepatocyte reserve but many vacuoles are found in the hindgut;

Grade 6: Very good condition with hepatic reserves. Intestinal cells are high and show absorption vacuoles. Pancreas acinis are well-structured with lots of zymogen.

Beside these grades, it is possible to determine if the larvae have recently fed. Recent feeding is indicated by caliciform cells in the foregut or vacuoles in the midgut and hindgut.

**Multivariate analysis approach**

Giving grades based on histology can be difficult because of differences between observers. A procedure based on simple questions and multivariate analysis is proposed to help determining the condition index of a larva (Tab. I). For each organ, the shape of the cell, nucleus, vacuoles and other particular point of interest (cytoplasm density, secretions, etc.) are coded, this represents 19 questions in the form. For each questions, different possible answers or categories are given. If the caliciform cells in the foregut are numerous, the answer will be CC3 from table I.

To achieve the multivariate analysis, the 19 questions, which are qualitative variables are transformed into 53 Boolean (0/1) categories. For the caliciform cells, 4 categories are given, absent (CC0), few (CC1), some (CC2) or numerous (CC3). If the caliciform cells are numerous, 1 will be given for CC3 and 0 for the other categories. The same procedure is applied for the other questions of the form.

Correspondence analysis suits this kind of categorical data (Benzécri, 1973). Because of barycentric projection, the clouds representing each larva and the one of the 53 Boolean histological categories can be plotted together. To help interpreting each cloud, cluster analysis was done separately on

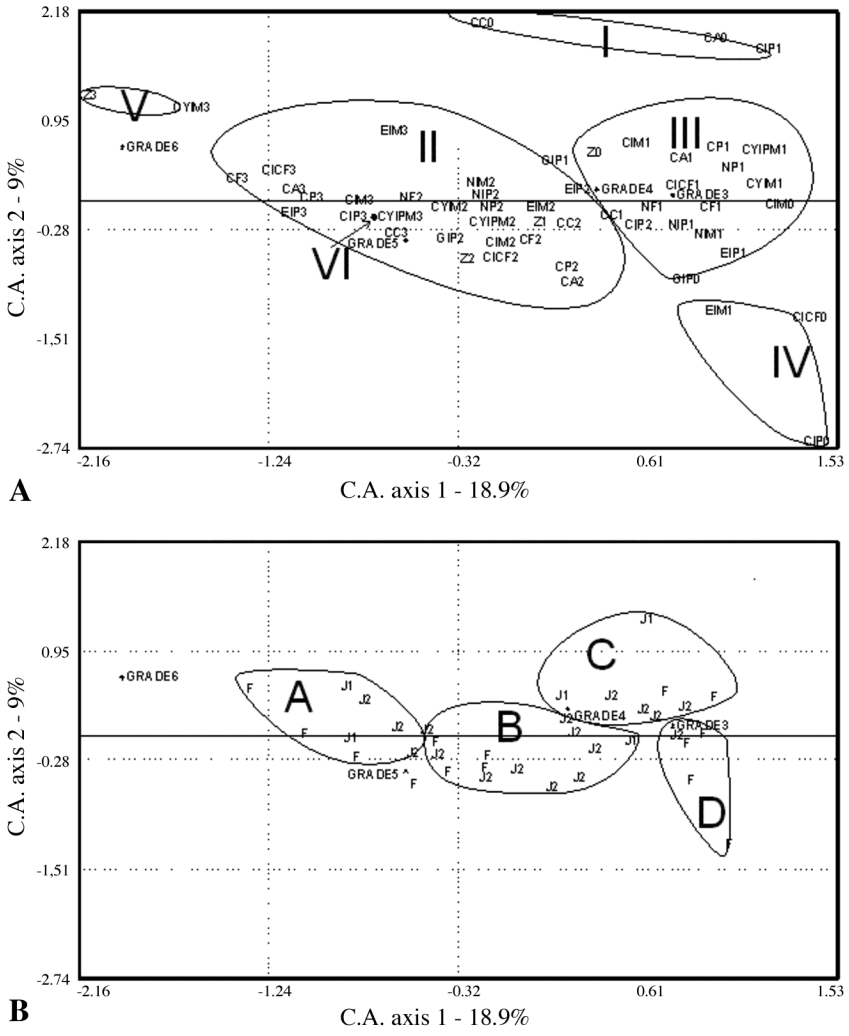


Figure 2. - Correspondence analysis on larval condition of *Pleuragramma antarcticum* determined by histology of liver, pancreas, foregut, midgut and hindgut. **A**: Factorial plane 1-2 of qualitative assessment larval condition using categories of tissues and cells (Tab. I). Histological grades (Grioche, 1998) were input as supplementary variables. **B**: Factorial plane 1-2 of fish sampled. J1 is for larvae of stations 85 or 86 in mid-January), J2 (station 72 on 31st of January) and F (station 53 at the beginning of February). *[Analyse factorielle de correspondance sur les conditions larvaires de* P. antarcticum *déterminées par l'étude histologique du foie, du pancréas, de l'estomac, de l'intestin grêle et du gros intestin. **A** : Plan factoriel des axes 1-2 des conditions larvaires estimées à partir des catégories histologiques des tissus et cellules (Tab. I). Les grades histologiques (Grioche, 1998) ont été mis en variables supplémentaires. **B** : Plan factoriel des axes 1-2 des larves étudiées. J1 correspond aux larves des stations 85 et 86 (mi-janvier), J2 (station 72 du 31 janvier) et F (station 53 du début février).]*

each cloud by taking multivariate scores of larvae and then scores of the 53 categories along the first three factorial axes (Koubbi *et al.*, 1991), which gather 36% of the whole information. Clustering was projected on an Euclidian distance matrix between larvae or the 53 categories using a complete linkage (the furthest neighbour) to maximize differences.

The histological grades given by direct observations are used as supplementary variables in the correspondence analysis to validate the use of the multivariate analysis approach.

**Diet**

For the fish larvae kept in 5% formalin, the gut contents were analysed using a stereomicroscope.

Gut contents from 55 fish were also analysed using a Scanning Electron Microscope (SEM) in order to identify diatoms and silicoflagellates when they were present. The fish larvae were rinsed in distilled water for two hours and put on a carbon tape attached to a metal stub (25 mm diameter). The gut of each individual was directly dissected on the metal stub. Specimens were dried, carbon-coated and observed with a LEO (438VP) scanning electron microscope. Genus identification was made according to Scott and Marchant (2004). The valve diameter of the Centric diatoms, the length and width of the Pennate diatoms and the diameter of the Silicoflagellates were measured using an image analyser LEO32. The length of the diatoms of the genus *Thalassiothrix* could not be measured because the cells in the gut were broken. Thus, for this genus, only the width was measured. The individual length of this genus is usually 420-5680 µm (Scott and Marchant, 2004).

The percentage of larvae with empty stomachs (percentage of vacuity) was assessed, and the prey frequency index f of the major taxonomic groups was calculated (Hureau, 1970). This index is f = n/N, where n is the number of fish larvae for a given prey and N is the number of full guts examined.

## RESULTS

### Multivariate analysis on larval condition

Correspondence analysis was based on the 53 histologic categories from 36 fish larvae. The percentages of variance explained by the first three axes were 18.86, 8.98 and 8.73%.

Four clusters of fish sampled were identified and plotted (Fig. 2B). They are spread along the first factorial plane except for a few extreme values. Figure 3 gives the percentage of larvae from each station per cluster. The larvae from mid-January are spread in all clusters except in cluster D. The larvae from station 72 (end of January) show few individuals in cluster D but most of them were in cluster B. Finally, the larvae from station 53 (February) are spread in all groups but mainly in cluster B. From these results it can be said that there seems to be a weak temporal pattern with an increase in the percentage of larvae in cluster D throughout time.

From the clustering of histologic categories, six groups were determined and also plotted on the factorial planes (Fig. 2A). The first factorial plane can be explained by a gradient from good to poor condition. Cluster V indicates the larvae in the best condition, with a high density of zymogen in the pancreas and a dense cytoplasm in the midgut. Cluster VI indicates a dense cytoplasm in the hindgut. Cluster II includes the larvae in good condition (Tab. II). Cluster III indicates some starvation (Tab. II). Cluster IV indicates severe starvation. The second factorial plane separates cluster IV from cluster I. Cluster I is characterised by signs of starvation.

From these results we can relate the groups of larvae to the groups of parameters. Table III shows that the first and second clusters of fish sampled (A and B) have most larvae in good condition. The cluster D contains most larvae in the poorest condition. Cluster C has larvae in medium condition.

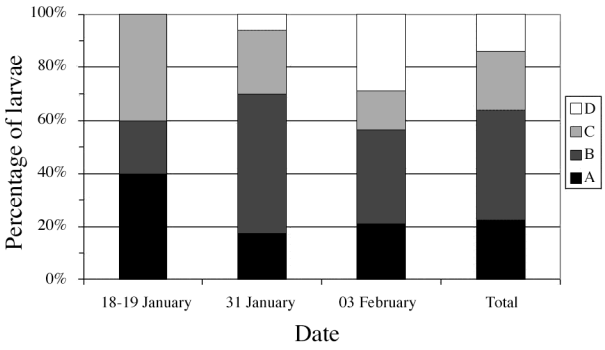


Figure 3. - Percentage by sampling date of *Pleuragramma antarcticum* larvae in groups A to D from the correspondence analysis of Fig. 2B. *[Pourcentage de larves de* P. antarcticum *par date d'échantillonnage dans les groupes A à D issus de l'analyse factorielle des correspondances.]*

Table III. - Relation between cluster of larval condition and cluster of fish samples in the correspondence analysis. Percentage of larvae sampled in each larval condition and fish cluster are given. *[Relation entre les groupes des conditions larvaires et les groupes issus de l'analyse factorielle des correspondances. Les pourcentages de larves par groupe de condition larvaire et par groupe de poissons sont donnés.]*

| Cluster of larval condition | Cluster of fish samples | | | |
|---|---|---|---|---|
| | A | B | C | D |
| V -Very good | *2.21* | 0 | 0 | 0 |
| VI- Very good | 0 | *0.39* | 0 | 0 |
| II- Good | 86.76 | *73.73* | 40.44 | 31.76 |
| III- Medium | 10.29 | 25.49 | *55.15* | 60.00 |
| I- Bad | 0.74 | 0 | *4.41* | 0 |
| IV- Very bad | 0 | 0.39 | 0 | *8.24* |

The plot of the histological grades according to Grioche (1998) and Boulhic (1991) shows a similar pattern to those based on our method.

Table II. - Characteristics of clusters II and III from the correspondence analysis. *[Caractéristiques des groupes II et III de l'analyse factorielle des correspondances.]*

| Cluster II | Foregut | Midgut | Hindgut | Liver | Pancreas |
|---|---|---|---|---|---|
| Cells | | | | | |
| Shape | | High cells | High cells | Large | Large |
| Cytoplasm density | | Full | Full | | |
| Nucleus | | Regular | Regular | Regular | |
| Vacuoles, secretions | Caliciform cells | | Vacuoles | | Zymogen |
| Intercellular cohesion | | Good | Excellent | Good | Good cohesion of acinis |

| Cluster III | Foregut | Midgut | Hindgut | Liver | Pancreas |
|---|---|---|---|---|---|
| Cells | | | | | |
| Shape | | | Cube-shaped | Few irregular | Irregular |
| Cytoplasm density | | Clear | Clear | | |
| Nucleus | | | Few irregular | Few irregular | Medium-good |
| Vacuoles, secretions | Few caliciform cells | | No vacuoles | | Zymogen absent |
| Intercellular cohesion | | Bad | Good | Bad | Bad |

**Copepod abundances**

Abundance range of copepods are from 12.8 to 15.3 individuals $m^{-3}$ in mid-January, 6.1 to 28.6 in late-January and from 5.3 to 8.9 in the beginning of February.

**Diet**

Thirty percent of the larvae had empty guts in January and nearly 40% in early February. Except for mid-January, more than 70% of the larvae had diatoms in their guts when the observations were made with a stereomicroscope.

The stereomicroscope showed that the most frequent prey were copepods and planktonic eggs. Copepods occurred in all the guts from mid-January. The frequency index of diatoms when using a stereomicroscope was underestimated in fish larvae guts (Fig. 4) since small cells are not easy to see.

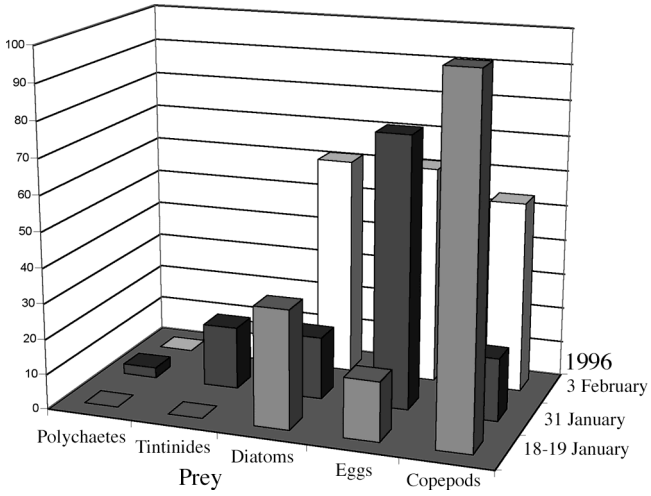


Figure 4. - Frequency index of gut contents of *Pleuragramma antarcticum* larvae from dissection for the main preys. *[Indice de fréquence des contenus stomacaux des larves de* P. antarcticum, *pour les proies principales.]*

With a SEM, only six fish larvae showed an empty gut (10.9% of 55 larvae). One genus of silicoflagellates and 15 genera of diatoms (11 Centric and 4 Pennate) were observed in gut contents (Tab. IV). The Pennate ones were the most frequent diatoms observed in the guts, especially the genera *Fragilariopsis* (71%) and *Thalassiothrix* (55%). Centric diatoms were well represented in the guts by the genera *Chaetoceros* (43%), *Thalassiosira* (37%) and *Coscinodiscus* (31%). The other genera represented less than 30% (Fig. 5).

The valve diameter of the Centrale diatoms varied between 13 µm (*Rhizosolenia* sp. and *Dactyliosolen* sp.) and 89 µm (*Coscinodiscus* sp.) (Fig. 6). The length of the Pennate diatoms varied from 28 to 41 µm (Fig. 6). The width of the Pennate diatoms varied between 5 µm (*Nitzschia* sp. and *Thalassiothrix* sp.) and 12 µm (*Achnanthes* sp.).

## DISCUSSION

**Condition index**

The grades according to the procedure of Grioche (2001) given by the observations and the results of the multivariate analysis based on a standard procedure with histologic categories are in accordance (Fig. 2). The multivariate analysis can be considered as a very useful tool to determine larval physiological condition as it can help to standardize the determination of grades with avoiding problems of giving grades by observers. We recommend this procedure in addition to direct grading.

From our results, most larvae were neither in excellent condition nor in bad condition (Tab. III). The larvae of cluster D were between grades 2 and 3, that is to say in severe starvation (Boulhic, 1991). Most larvae of cluster C were mainly in grade 3, showing high starvation with renutrition.

| | | 18-19 January | 31 January | 3 February | Total |
|---|---|---|---|---|---|
| Centric diatoms | *Actinocyclus* sp. | - | 33.33 | 6.67 | 22.45 |
| | *Asteromphalus* sp. | 25.00 | 23.33 | 33.33 | 26.53 |
| | *Chaetoceros* sp. | - | 40.00 | 60.00 | 42.86 |
| | *Corethron* sp. | 25.00 | 6.67 | 6.67 | 8.16 |
| | *Coscinodiscus* sp. | 50.00 | 23.33 | 40.00 | 30.61 |
| | *Dactyliosolen* sp. | - | - | 6.67 | 2.04 |
| | *Eucampia* sp. | - | 10.00 | - | 6.12 |
| | *Odontella* sp. | - | - | 13.33 | 4.08 |
| | *Porosira* sp. | - | 3.33 | 13.33 | 6.12 |
| | *Rhizosolenia* sp. | - | 30.00 | 20.00 | 24.49 |
| | *Thalassiosira* sp. | - | 33.33 | 53.33 | 36.73 |
| Pennate diatoms | *Achnanthes* sp. | - | - | 13.33 | 4.08 |
| | *Fragilariopsis* sp. | 100.00 | 66.67 | 73.33 | 71.43 |
| | *Nitzschia* sp. | - | - | 6.67 | 2.04 |
| | *Thalassiothrix* sp. | - | 60.00 | 60.00 | 55.10 |
| Silicoflagellates | *Dictyocha* sp. | 25.00 | 16.67 | 13.33 | 16.33 |

Table IV. - Frequency index (%) of diatoms and silicoflagellates in gut contents of *Pleuragramma antarcticum* larvae from SEM observations, at each sampling date. *[Indice de fréquence (%) des diatomées et silicoflagéllés dans les tubes digestifs des larves de* P. antarcticum *observés au microscope électronique à balayage par date d'échantillonnage.]*

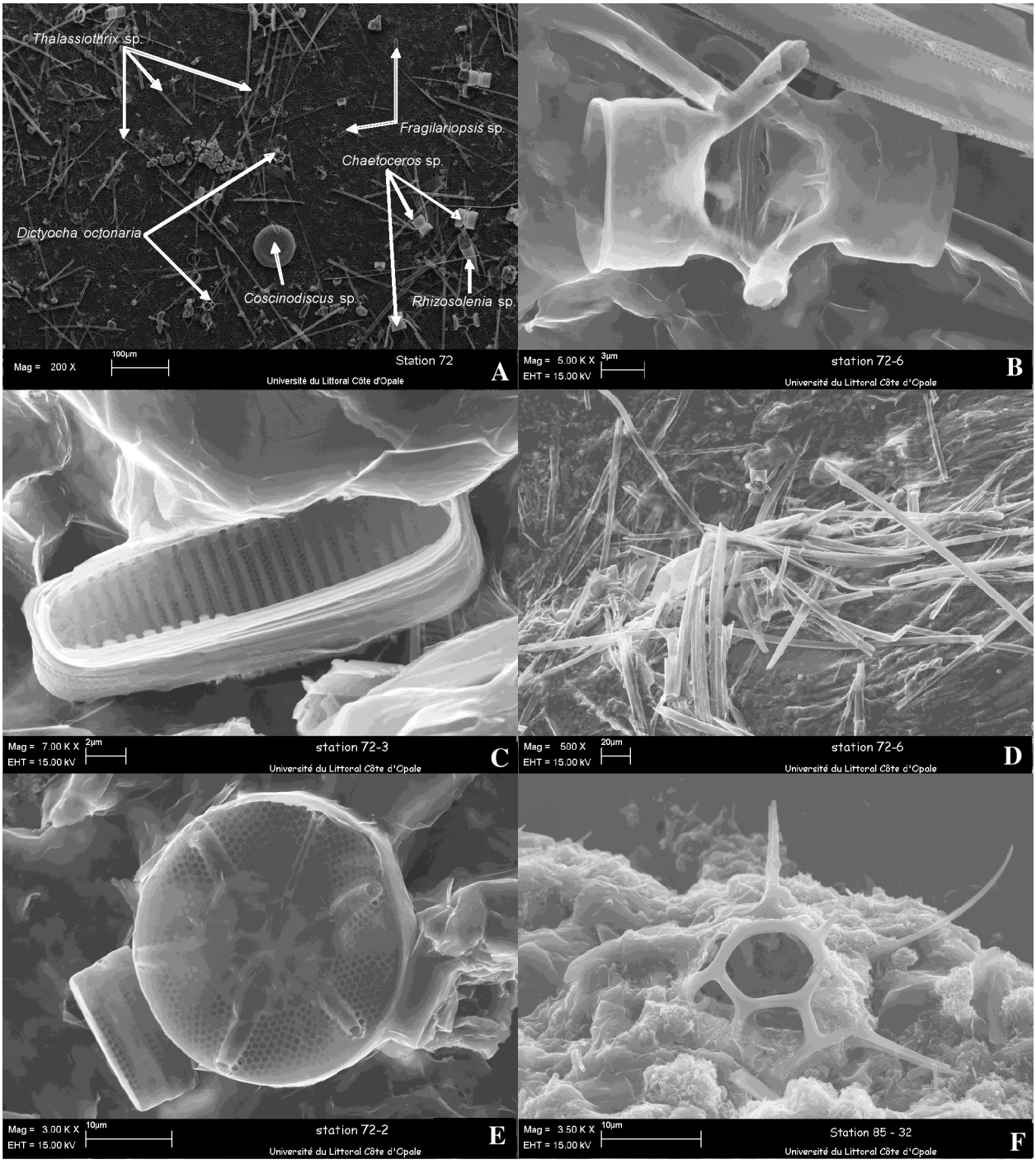


Figure 5 - SEM pictures of the main genera of diatoms found in the gut of *Pleuragramma antarcticum* larvae. **A**: Gut contents for a larvae caught at station 72; **B**: *Chaetoceros* sp. - station 72; **C**: *Fragilariopsis* sp. - station 72; **D**: Several peaces of *Thalassiothrix* sp. - station 72; **E**: *Asteromphalus* sp. - station 72; **F**: *Dictyocha* sp. - station 85. *[Photographies obtenues par microscopie électronique à balayage, des principaux genres de diatomées trouvés dans les tubes digestifs des larves de* P. antarcticum*; **A** : Contenus des tubes digestifs de larves échantillonnées à la station 72 ; **B** :* Chaetoceros *sp. station 72 ; **C** :* Fragilariopsis *sp. - station 72 ; **D** :Différentes parties de* Thalassiothrix *sp. - station 72; **E** :* Asteromphalus *sp. - station 72 ; **F** :* Dictyocha *sp. - station 85.]*

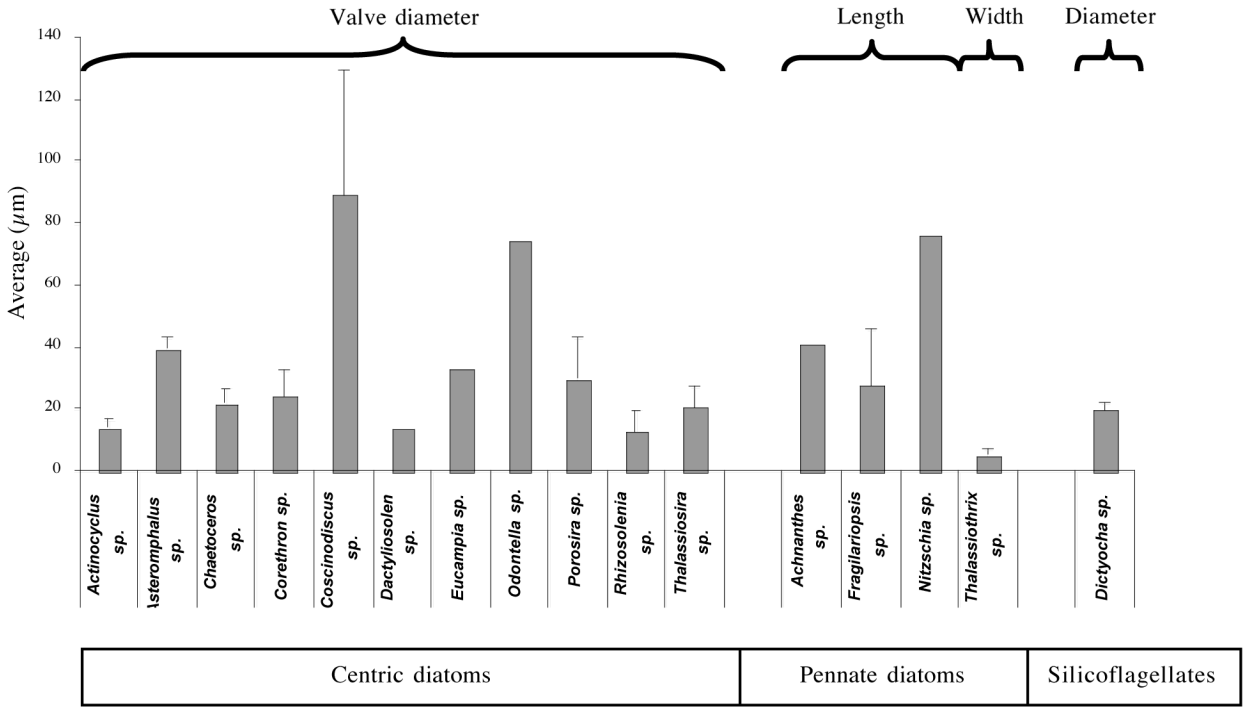


Figure 6. - Sizes of the main phytoplanktonic preys found in the gut content of *Pleuragramma antarcticum* larvae. Measurements obtained from scanning electron microscope observations. *[Tailles des principales espèces phytoplanctoniques trouvées dans les tubes digestifs des larves de* P. antarcticum. *Les mesures ont été obtenues par observations au microscope électronique à balayage.]*

The larvae of cluster B, between grade 4 and 5, showed recent starvation with good renutrition. The larvae of cluster A were in good or very good condition corresponding to grade 5 or grade 6. From our observations, the organs first affected by starvation were the midgut and the liver and the least affected seemed to be the pancreas and the hindgut.

There was no sign of accumulation of reserves (glycogen), especially in the liver, even if cells were generally high and tissues mainly in good condition for most of the larvae. There were few vacuoles in the midgut and lots of them were observed in the hindgut for the larvae in the best condition. The zymogen concentration in the pancreas was variable among the larvae. These results differed from similar studies in temperate seas (Boulhic, 1991; Grioche, 1998) showing high accumulation of hepatic reserves for some larvae.

**Diet**

The guts of the *P. antarcticum* larvae from the end of January and the beginning of February had low copepod frequency index. At that period larvae fed essentially on diatoms and eggs, while in mid-January, this index is high for copepods. Tintinnids and polychaetes frequency index were the smallest ones with maximal values at the end of January.

One of the most puzzling results is the high amount of diatoms in the gut contents of these larvae as shown by the use of SEM (Fig. 4). Marine microalgae are widely used in first-feeding of marine fish larvae (Jones *et al.*, 1981; Lubzens, 1987; Naas *et al.*, 1992; Reitan *et al.*, 1993). According to many authors, when fish larvae were reared in tanks, the presence of microalgae improved their growth and survival (Moffat, 1981; Naas *et al.*, 1992; Reitan *et al.*, 1997; Cahu *et al.*, 1998; Lazo *et al.*, 2000; Rønnestad *et al.*, 2003). In this study, when copepods were less abundant or highly variable, the larvae fed essentially on diatoms. Their condition, as observed later on during the summer period, seemed to be poorer than in mid-January. It can be hypothesized that during the phytoplanktonic bloom in summer, diatoms probably stabilize the nutritional conditions of larvae, which can wait for a better time to feed on copepods, their most common prey (Hubold, 1985; Kellermann, 1987; Hubold and Ekau, 1990). Phytoplankton, especially diatoms, may provide a direct supply of nutrients (Moffat, 1981) and free amino acids, which are contained in large amounts in diatoms (Hammer *et al.*, 1981; Admiral *et al.*, 1986) and which played an important role in energy production and protein synthesis (Fyhn, 1993). Moreover, the presence of phytoplankton would also tend to affect the microbial community of the water, and thus, larval gut microflora (Gatesoupe, 1991; Skjermo and Vadstein, 1993; Oivind *et al.*, 1994). Lazo *et al.* (2000) suggested that fish larvae in early development had a lack of digestive enzymes. Cahu and Zambonino-Infante (1994) assumed that the presence of phytoplankton in the diet of fish larvae can influence the activity of digestive enzymes, such as trypsin. Zambonino-Infante and Cahu

(1994) reported that the trypsin was activated when the diet was supplemented with a mixture of free amino acids. As diatoms contain large amounts of free amino acids, it can be hypothesized that the amino acids released could be responsible for trypsin activation in the larvae. Moreover, many compounds, which are natural constituents of phytoplankton or zooplankton (Dabrowski and Ruseick, 1983), such as betaine, inosine 5-monophosphate and amino acids, might act as a chemical stimulant to the feeding behaviour of fish larvae (Lazo *et al.*, 2000). It could be possible that in extreme conditions as in the Antarctic, *P. antarcticum* larvae need to feed on phytoplankton in order to get a better digestion and assimilation of large prey such as copepods.

This means that these larvae are omnivorous. We do not know if the feeding on diatoms only is better than feeding on copepods like cyclopoids – their most common preys (Hubold, 1985; Kellermann, 1987; Hubold and Ekau, 1990). It seems that the best diet is a mixture of diatoms and copepods. Eggs, tintinnids and other preys such as polychaetes are probably linked to a shift in the diet when copepods are not sufficiently abundant.

**Lipid storage**

The larvae we studied were from the age class 0 that hatched around the end of November and December. We tried to assess whether the high pelagic production in summer is favourable to the accumulation of carbohydrates or lipids reserves in the fish to face the winter or not. From our results there are no signs of accumulation or vacuolisation in the liver. We only showed differences in size of liver cells indicating good condition for some larvae. Nevertheless, the lack of vacuolisation in the liver may be due to the particular lipid storage of this species.

*P. antarcticum* is unique in having large subcutaneous or intermuscular lipid sacs along the body whereas the other fish mainly store lipid in hepatocytes (Eastman, 1990). In *P. antarcticum* lipid deposits are mainly used for buoyancy rather than as an energy reserve for the winter season (DeVries and Eastman, 1978). Lipids sacs are mainly made of triacylglycerols that can be used, if necessary for metabolism. Do larval *P. antarcticum* attain neutral buoyancy?

If the larvae do not accumulate reserves, it may mean that the winter zooplankton abundance is sufficient for survival. Hubold and Hagen (1997) showed that in summer the larvae of this species do not accumulate lipids and that the larvae adapt their diets according to the dominant copepods present.

## CONCLUSION

The study of *P. antarcticum* diet during summer plankton blooms shows that these larvae are not simply carnivorous but omnivorous. A diet based both on zooplankton and phytoplankton may be better than a diet only based on phytoplankton. The summer feeding does not seem to favour accumulation of reserves for the winter. Most energy was probably directed to larval growth. That means that larvae have to feed during winter in order to survive. Moreover, species with low metabolism (Wöehrmann, 1998) and larger larvae may have more chance to survive in winter, as for temperate species.

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